

#### **SAFETY CONSIDERATIONS**

- The optogenetics setup uses a 12-24V power supply. Before touching any part of the circuit board or wires make sure that the power supply is off and unplugged.
- Do not touch the LEDs or wires while the system is plugged into the power supply.
- Avoid staring directly at the LED lights while they are on.

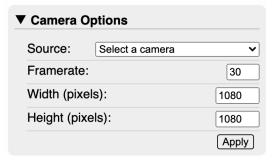
## Starting the program

- 1. Make sure the camera is connected to the USB port.
- 2. Make sure the IR and LED lights are plugged into the board but connect the the power yet.
- 3. Launch FlyRig.exe if using executable.
- 4. Close the tab on the right side of the screen by clicking on the X (red circle below).



## **Setting up the Camera**

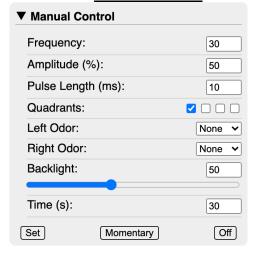
1. Click on Camera Options. It should give you a window that looks like this:



- 2. Select the USB camera under the Source tab.
- 3. Set the Framerate (frames per second) to 30
- 4. Set the Width and Height to desired pixels. The maximum for the included camera is 1920 x 1080.
- 5. When the camera connects the screen with change from gray to black. You will not see an image until you turn on the Backlight (next step).

## **Turning on the Backlight**

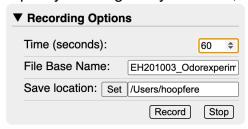
1. Click on the Manual Control tab.



2. The <u>Backlight</u> slider allows you to set the intensity of the backlight (from 0 – 100%). **Set it at 100% to begin with.** The background of the image should brighten. You won't be able to see the light coming from the backlight because it is in the infrared wavelength (IR), which we and the flies can't see.

## Making a video recording

1. To specify the length of your video, click on the Recording Options tab.



- 2. Set the Time (in seconds) of your recording.
- 3. Set the name of your video in <u>File Base Name</u>. The video filename will start with this name. The video
- 4. Specify where you want to save your video in <u>File Base Name</u>. It should open a browser window. Select the folder where you want to save your videos.
- 5. To start your recording click the <u>Record</u> button. While the video is recording, you can manually control the LEDs (see Manual Control section).
- 6. The videos are encoded as mp4 files.
- 7. The filenames of each recording are automatically appended as follows to prevent overwriting files.

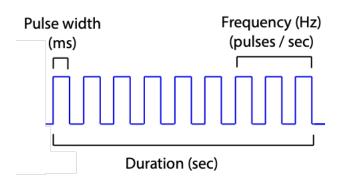
Filename\_Year\_Month\_DayTHour\_Min\_Sec\_msecZ

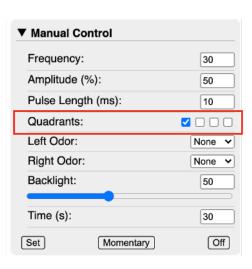
#### **Manual Control**

This tab allows you to manually control the LED lights. Changing the intensity or frequency of light that you use for optogenetic stimulation can cause a corresponding change in the activity of the neurons you're activating.

You can set the following parameters of stimulation:

- Frequency of stimulation (Hz): adjusts the frequency of pulses per sec.
- Amplitude (%): adjusts the intensity of light from 0 to 100% max intensity
- Pulse length (ms): this is the duration of each pulse.
- **Time** (sec): this is the duration of the stimulation (how long you stimulate)
- Quadrants: Which set of LEDs to activate.





# Option 1: Instant control of the light

- 1. Clicking on the <u>Momentary</u> button will turn on the light. This is useful for giving instantaneous stimulations.
- 2. Set the <u>Frequency</u>, <u>Amplitude</u>, <u>Pulse length</u>.
- 3. Select the proper <u>Quadrants</u> to activate. If the board has only one LED driver, click on the leftmost box.
- 4. Set the Time (seconds) of the stimulation.
- 5. Click on the Momentary button (while recording or not) to deliver the light. Hold down the button as long as you want to deliver light.

#### Option 2: Set duration of light stimulation

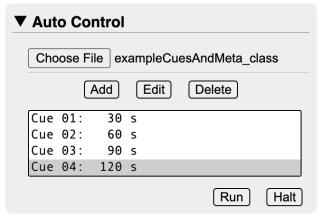
This is useful for repeating the same stimulation.

- 1. Set the Frequency, Amplitude, Pulse length, and Quantrants.
- 2. Set the Time (seconds) of the stimulation.
- 3. The light will start as soon as you click on the <u>Set</u> button. This can be done at any time, including during a recording.
- 4. To turn off the light in the middle of a stimulation click on the Off button.

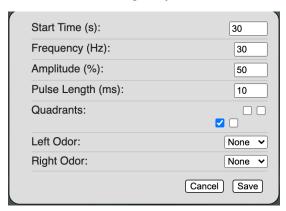
#### **Auto Control**

This setting allows you to pre-program a set of light stimulations and experimental metadata. This can be useful if you want to give repeated stimulation protocols during several experiments.

- 1. Click on the Auto Control tab.
- 2. Each <u>Cue</u> is a separate "time period" during your experiment. You can either set the light to ON or OFF during these periods.
- 3. To get you started, you can load an example CSV file that will pre-load some Cues into the program.
- 4. To do so, click on Choose File.
- 5. You'll find a file called "exampleCuesAndMetadata\_class.csv" on the desktop of the computer.
- 6. This file will load an experiment that does the following:
  - a. Cue 1: No light for 30 seconds (Time 0 30 sec)
  - b. Cue 2: 30 Hz light at 50% intensity for 30 seconds (Time 30 60 sec)
  - c. Cue 3: 30 seconds of no light (Time 60 90 sec)
  - d. Cue 4: 30 Hz light at 50% intensity for 30 seconds (Time 90 120 sec)
- 7. You can Add, Edit or Delete Cues.



8. Add and Edit will give you this window.



- 9. The <u>Start Time</u> indicates when during the recording (i.e., elapsed time) the cue should start. Make sure it doesn't overlap with other cues.
- 10. Once you have your Cues set, you will want to specify the file path and name of your recording (Recording Options tab).
- 11. Clicking on the <u>Run</u> button of the <u>Auto Control</u> tab will start a recording and the light stimulation experiment you programmed into Auto Control.